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**"Analysis of Nearest Neighbor Interactions in the Pyrimidine
Triple Helix Motif by Affinity Cleaving"**

by

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Pasadena, CA**

June 1, 1991

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Analysis of Nearest Neighbor Interactions in the Pyrimidine Triple Helix Motif by Affinity Cleaving.

Affinity cleaving studies provide evidence that nearest neighbor interactions affect the relative stabilities of triple helices. Several groups of target duplexes were synthesized with a central base triplet held constant and the adjacent 3' and 5' triplets systematically varied. By incorporating a thymidine residue with the DNA-cleaving moiety EDTA in the third strand, the relative stabilities of Hoogsteen base triplets T·AT and C+GC as well as the newly discovered base triplets G·TA, Z·TA, and D₃·CG were assessed in the context of different sequences. The T·AT triplet was shown to be relatively insensitive to substitutions in either the 3' or 5' directions, while relative stabilities of triple helices containing C+GC triplets decreased as the number of adjacent C+GC triplets increased. Triple helices incorporating a G·TA interaction were most stable when this triplet was flanked by two T·AT triplets, and were most adversely affected when a C+GC triplet was placed in the adjacent 5' direction. In contrast, complexes containing a D₃·TA interaction were destabilized when the adjacent 3' position was occupied with a C+GC triplet. The D₃·CG interaction displayed the same binding preferences. New guidelines for targeting sequences containing pyrimidine base pairs have been developed.



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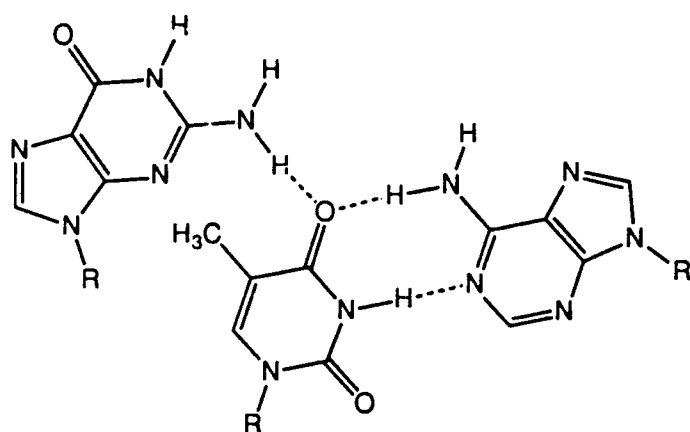
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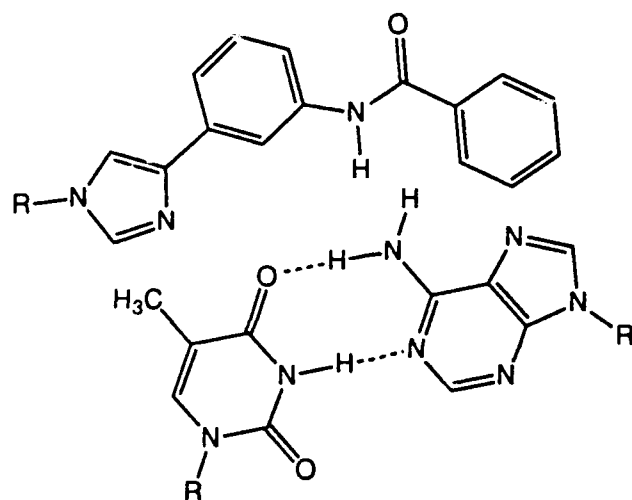
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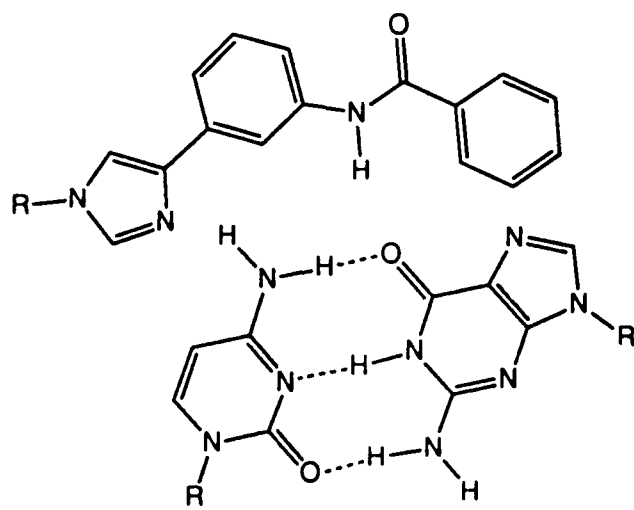
Oligonucleotide-directed sequence specific recognition of double helical DNA is length and sequence composition dependent. In order to dissect the relative importance of nearest neighbor interactions on triplet stabilities, binding by oligonucleotides of different sequence composition has been characterized by affinity cleaving. Several groups of target duplexes were synthesized with a central base triplet held constant and the adjacent 3' and 5' triplets systematically varied. By incorporating a thymidine residue with the DNA-cleaving moiety EDTA in the third strand, the relative stabilities of Hoogsteen base triplets T·AT and C+GC as well as the newly discovered base triplets G·TA, Z·TA, and D₃·CG were assessed in the context of different sequences. The T·AT triplet was shown to be relatively insensitive to substitutions in either the 3' or 5' directions, while relative stabilities of triple helices containing C+GC triplets decreased as the number of adjacent C+GC triplets increased. Triple helices incorporating a G·TA interaction were most stable when this triplet was flanked by two T·AT triplets, and were most adversely affected when a C+GC triplet was placed in the adjacent 5' direction. In contrast, complexes containing a D₃·TA interaction were destabilized when the adjacent 3' position was occupied with a C+GC triplet. The D₃·CG interaction displayed the same binding preferences. New guidelines for targeting sequences containing pyrimidine base pairs have been developed.



G•TA base triplet



D₃•TA base triplet



D₃•CG base triplet

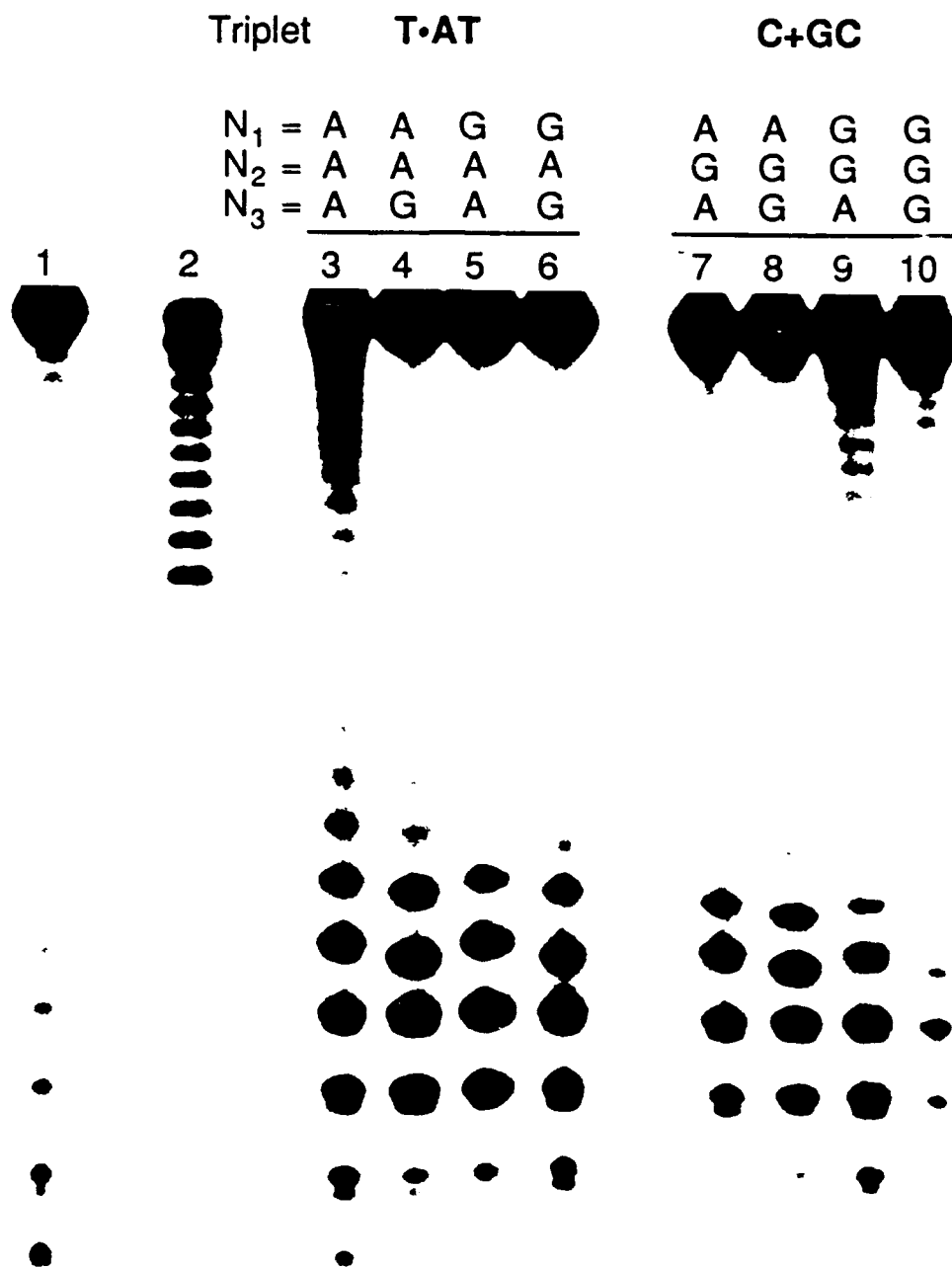
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5'- T T T T T [*] T T T T C T T T T T T T -3'	2
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5'- T T T T T [*] T T C T C T T T T T T T -3'	4
5'- T T T T T [*] T T T C T T T T T T T T -3'	5
5'- T T T T T [*] T T T C C T T T T T T T -3'	6
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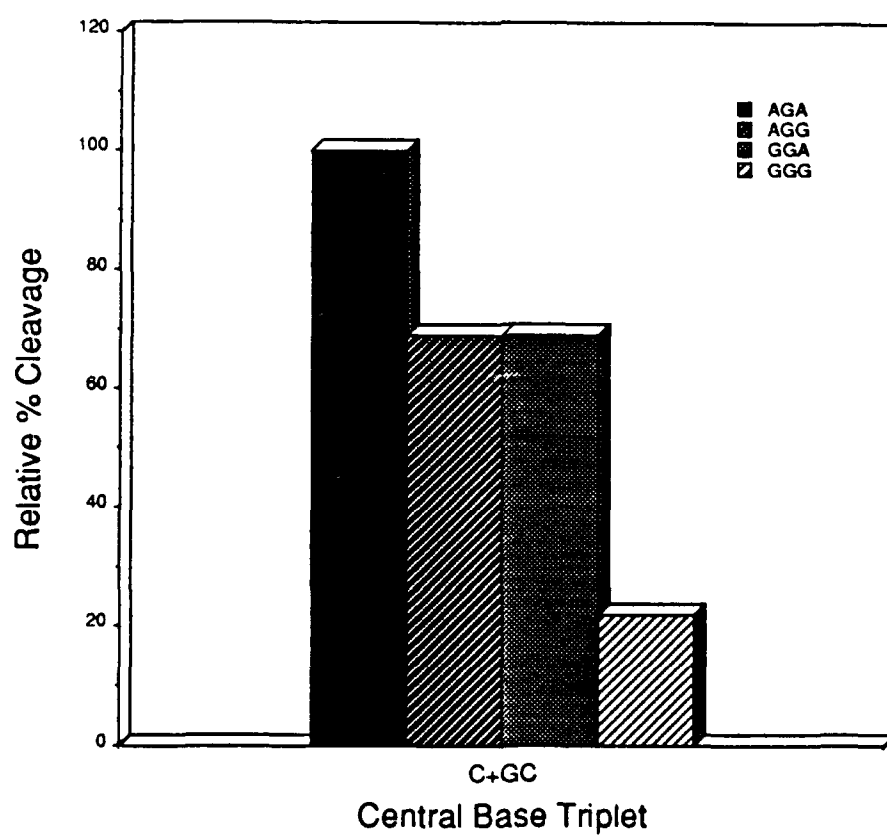
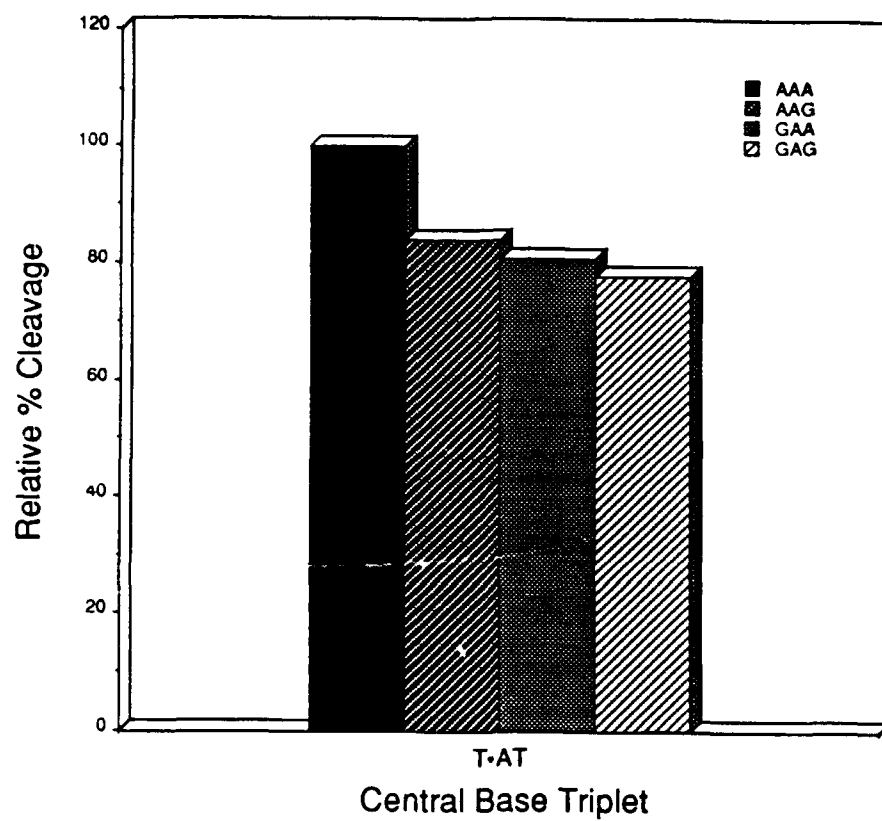
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3'- G G G G G G G G G G T T T T T N₆ N₅ N₄ T T T T T T A A A A A -5'



N ₁ N ₂ N ₃ = AAA 9a	N ₄ N ₅ N ₆ = TTT 9b
N ₁ N ₂ N ₃ = AAG 10a	N ₄ N ₅ N ₆ = CTT 10b
N ₁ N ₂ N ₃ = GAA 11a	N ₄ N ₅ N ₆ = TTC 11b
N ₁ N ₂ N ₃ = GAG 12a	N ₄ N ₅ N ₆ = CTC 12b
N ₁ N ₂ N ₃ = AGA 13a	N ₄ N ₅ N ₆ = TCT 13b
N ₁ N ₂ N ₃ = AGG 14a	N ₁ N ₅ N ₆ = CCT 14b
N ₁ N ₂ N ₃ = GGA 15a	N ₄ N ₅ N ₆ = TCC 15b
N ₁ N ₂ N ₃ = GGG 16a	N ₄ N ₅ N ₆ = CCC 16b





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 5'- T T T T T ^{*} T C T T T T T T T -3' 19
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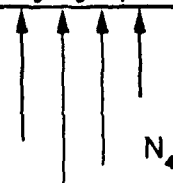
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 3'- G G G G G G G G G G T T T T T T N₆N₅N₄T T T T T A A A A A -5'



N ₁ N ₂ N ₃ = ATA	25a	N ₄ N ₅ N ₆ = TAT	25b
N ₁ N ₂ N ₃ = ATG	26a	N ₄ N ₅ N ₆ = CAT	26b
N ₁ N ₂ N ₃ = GTA	27a	N ₄ N ₅ N ₆ = TAC	27b
N ₁ N ₂ N ₃ = GTG	28a	N ₄ N ₅ N ₆ = CAC	28b

5'- T T T T T ^{*} T T D₃ T T T T T T T -3' 29
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 3'- G G G G G G G G G G T T T T T T N₆N₅N₄T T T T T A A A A A -5'



N ₁ N ₂ N ₃ = ATA	25a	N ₄ N ₅ N ₆ = TAT	25b
N ₁ N ₂ N ₃ = ATG	26a	N ₄ N ₅ N ₆ = CAT	26b
N ₁ N ₂ N ₃ = GTA	27a	N ₄ N ₅ N ₆ = TAC	27b
N ₁ N ₂ N ₃ = GTG	28a	N ₄ N ₅ N ₆ = CAC	28b
N ₁ N ₂ N ₃ = ACA	33a	N ₄ N ₅ N ₆ = TGT	33b
N ₁ N ₂ N ₃ = ACG	34a	N ₄ N ₅ N ₆ = CGT	34b
N ₁ N ₂ N ₃ = GCA	35a	N ₄ N ₅ N ₆ = TGC	35b
N ₁ N ₂ N ₃ = GCG	36a	N ₄ N ₅ N ₆ = CGC	36b

Triplet

I•TA

G•TA

D₃•TAD₃•CG

N₁= A A G G
N₂= T T T T
N₃= A G A G

A A G G
T T T T
A G A G

A A G G
T T T T
A G A G

A A G G
C C T T
A G A G

1

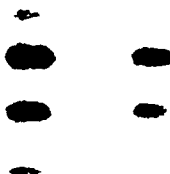
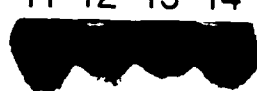
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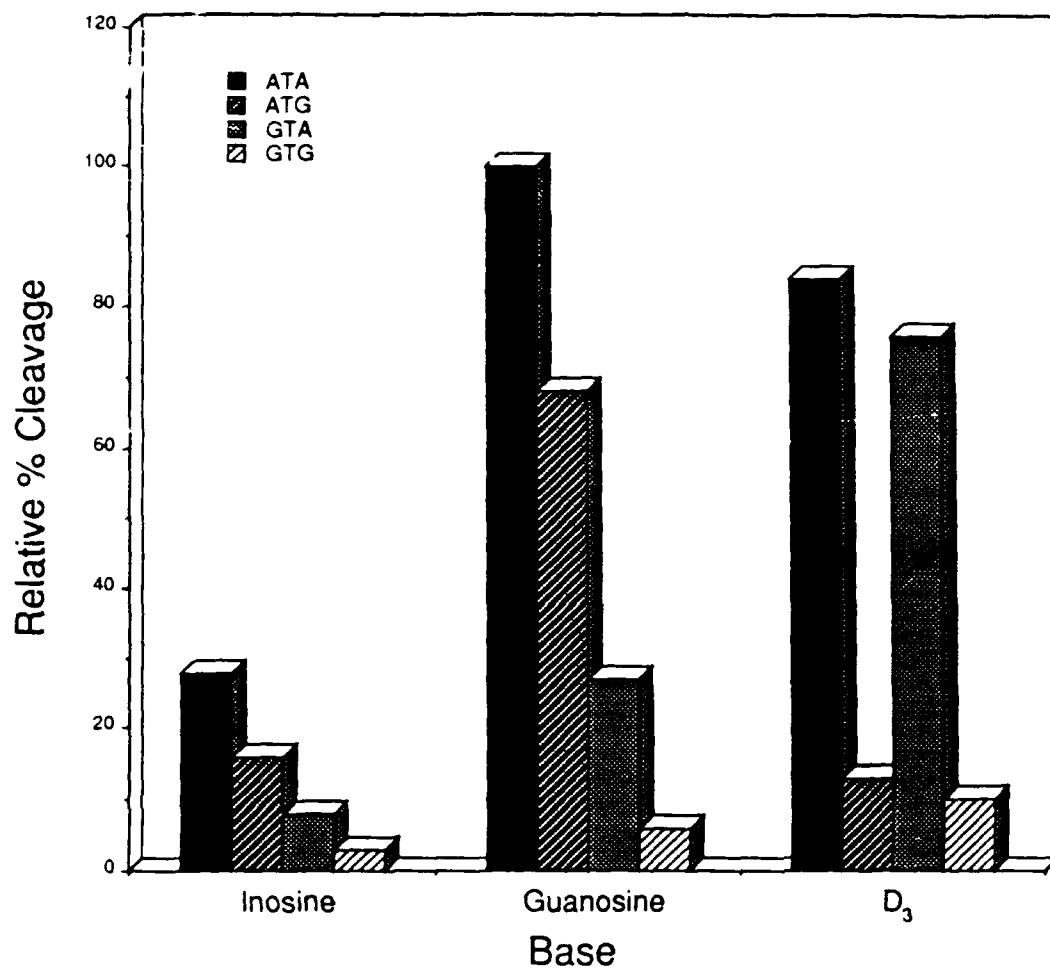
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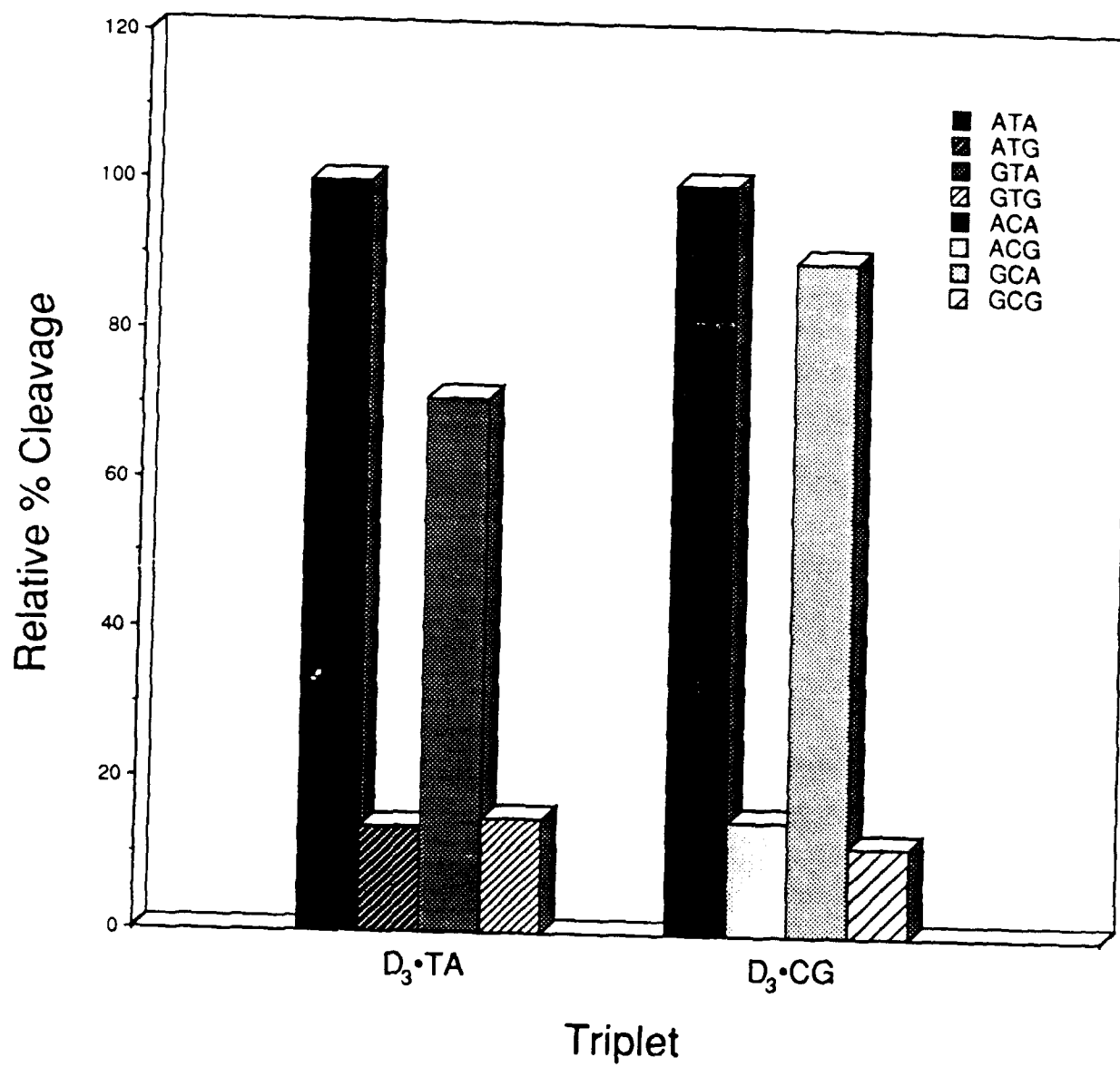
7 8 9 10

11 12 13 14

15 16 17 18







Target Sequence	G	D ₃
5'- ATA -3'	+++	+++
ATG	++	+
GTA	+	+++
GTG	-	-
5'- ACA -3'	-	+++
ACG	-	+
GCA	-	+++
GCG	-	-